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Chemoselective Attachment of Biologically Active Proteins to Surfaces by Native Chemical Ligation

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ABSTRACT

The present work describes our ongoing efforts towards the creation of micro and nano-scaled ordered arrays of protein covalently attached to site-specific chemical linkers patterned by different microlithographic techniques. We present a new and efficient solid-phase approach for the synthesis of chemically modified long alkyl-thiols. These compounds can be used to introduce chemoselective reacting groups onto silicon-based surfaces. We show that these modified thiols can be used for creating nano- and micrometric chemical patterns by using different lithographic techniques. We show that these patterns can react chemoselectively with proteins which have been recombinantly modified to contain complementary chemical groups at specific positions thus resulting in the oriented attachment of the protein to the surface.

INTRODUCTION

Many experimental techniques in biology and biophysics, and applications in diagnosis and drug discovery, require proteins immobilized on solid substrates. Most of the available methods, however, rely on non-specific adsorption, or on the random cross-linking of proteins to chemically reactive surfaces. In both cases the protein is attached to the surface in random orientations. The use of recombinant affinity tags addresses the orientation issue. However, in most cases the interactions of the tags are reversible and therefore not stable over the course of subsequent assays or require large mediator proteins. Covalent attachment and orientation of a protein to a solid support requires two unique and mutually reactive groups on the protein and the support surface. The reaction between these two groups should be highly chemoselective, thus behaving like a molecular '*velcro*'.

RESULTS AND DISCUSSION

Most of the methods suitable for the chemoselective attachment of proteins to surfaces are based on ligation methods originally developed for the synthesis, semi-synthesis and selective derivatization of proteins by chemical means (see reference [1] for a complete review). These methods involve the derivatization of the protein with a unique chemical group at a defined position, which will later react chemoselectively with a complementary group previously introduced onto the surface. Here, we describe the creation of micro and nano-scaled ordered arrays of protein covalently attached by native chemical ligation [2] to Cys-containing linkers patterned with different micro- and nanolithographic techniques.

Derivatization of Si-based surfaces with Cys-containing linkers

In the present work, we have developed an efficient solid-phase approach for the rapid

synthesis of chemically modified long alkane thiols (Figure 1A). These modified thiols can be easily used to chemically derivatized Si-based surfaces (i.e. SiO_2 wafers and glass), previously treated with acryloyl-containing silanes, with different chemical groups. Among others, the introduction of a Cys moiety allows the selective reaction with C-terminal α-thioester proteins (through native chemical ligation) [2], the amino-oxy group allows the reaction of carbonyl-containing proteins (which can be generated by mild oxidation of glycoproteins) and the tri-(ethylenglycol) (TEG) prevents non-specific adsorption. We have shown that these chemically modified thiols can be used to generate nano- and micrometric chemical patterns on Si-based surfaces by commercial DNA microarrayers [3], micro-contact printing [4] and dip-pen nanolithography techniques [5-6] (Figure 1B).

Chemoselective attachment of α-thioester proteins on chemically patterned surfaces

We used the N-terminal SH3 domain of the c-Crk adaptor protein as a model for testing the chemoselective attachment of α-thioester proteins (Figure 1C) to chemically patterned Cys-containing surfaces through its C-terminus by native chemical ligation [2]. The SH3 protein was cloned into an intein expression vector [7] and expressed in *E. coli*. After purification of the

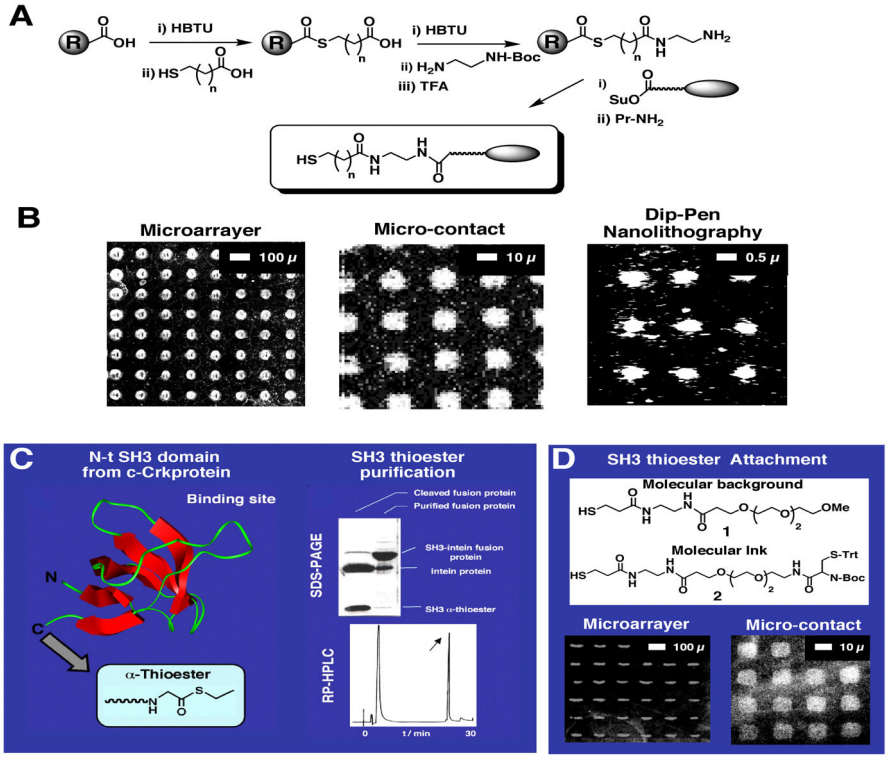

Figure 1. A. Synthetic scheme for the synthesis of chemically modified thioalkanes. The solid ellipsoid represents different groups that can be introduced onto the surface. **B**. Chemical templates of Cys-containing thiols produced on SiO_2 by different techniques. Epifluorescence image after reacting the surfaces with a thiol-reactive fluoreceine dye **C**. Expression and purification of an SH3 α-thioester protein. **D**. Deposition of the SH3 α-thioester on a Cys template produced by different techniques. Epifluorescence image of a chemically patterned surface using a Cys-containing linker (see inset) and then incubated with the α-thioester SH3 protein domain. After washing the unbound protein, the functional SH3 domain was detected by fluorescence after incubating the surface with a fluoresceine-tagged SH3 ligand.

SH3-intein fusion protein by affinity chromatography, the correpsonding α-thioester protein domain was generated by cleavage with EtSH in PBS at pH 7.2. The chemical template was produced by either micro-contact printing or comercially microarrayers on SiO_2 previously treated with an acrylyl-containg silane using compound **2** as ink (Figure 1D inset). The surface outside of the patterned area was then capped with TEG-containing thiol **1** to prevent any non-specific adsorption of the protein. The freshly obtained SH3 α-thioester protein domain was reacted with the corresponding surfaces in PBS at pH 7.2 buffer containing 5% EtSH overnight at room temperature. Once the ligation reaction was finished, the surface was extensively washed with PBS to remove any protein not covalently attached to the surface. In order to check the presence of the SH3 domain in the patterned areas, the protein-derivatized surfaces were incubated with a fluoresceine-tagged high-affinity ligand for the SH3 domain in PBS for 30 min. After washing with PBS the unbound ligand the surfaces were imaged with a confocal microscope. The results (Figure 1D) showed that the protein was preferentially covalently bound to the Cys-containing chemically patterned areas. Furthermore, the fact that the attached protein was able to recognize its ligand meant that the ligation process did not negatively affect the native folded state of the protein.

CONCLUSIONS

We have shown that the highly selective native chemical ligation reaction can be used in combination with several lithographic techniques for the creation of oriented biologically active protein arrays. We have also developed an efficient and highly modular chemical approach for the rapid synthesis of modified alkane thiols which can be used for the chemoselective attachment of proteins as well as surface passivation. Future work will involve the development of a totally novel and generic approach for the chemoenzymatic and photoswitchable attachment of proteins to surfaces by using protein trans-splicing units.

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